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DIFFERENCES IN PROTEIN CONTENTS AMONG FIBROBLAST CELL LINES GROWN IN MONOLAYER CULTURES

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ABSTRACT

The changes in cell protein values of cat kidney and McCoy's human synovial cell lines were determined during various phases of monolayer growth in a lactalbumin hydrolyzate - 10% calf serum medium. The McCoy's cell protein values ranged from 230 x 10^{-6} to 574 x 10^{-6} µg/cell, whereas the cat kidney cell values ranged from 131 x 10^{-6} to 367 x 10^{-6} µg/cell. Maximum values for each cell line were recorded during a short period in the lag phase of growth. Essentially constant minimum values were observed after the cultures reached a slowly proliferating, densely populated, post-confluent state. For this reason, the characteristic protein values reported for each cell line were determined during the latter part of the logarithmic growth phase.

An effect of composition of growth medium on cell protein values was observed. When cultured in a serum-free chemically defined medium, the protein values during the latter part of the logarithmic growth period averaged 281 (±28) x 10^{-6} µg/cell for McCoy's cells, 171 (±18) x 10^{-6} µg/cell for L cells, and 134 (±18) x 10^{-6} µg/cell for cat kidney cells. On the other hand, when grown in a lactalbumin hydrolyzate - 10% calf serum medium, the protein values averaged 249 (±20) x 10^{-6} µg/cell for McCoy's cells, 278 (±35) x 10^{-6} µg/cell for L cells, and 139 (±33) x 10^{-6} µg/cell for cat kidney cells.

It is believed that these measurements may serve, under suitable standardized conditions, as a method for the characterization of cell lines.

I. INTRODUCTION

Studies on the protein contents of mammalian cells cultured in vitro have been described by Oyama and Eagle,[1] by Salzman,[2] by Swaffield and Foley,[3] and by Miedema and Kruse.[4] The present report is an extension of their work with a view toward application of such data to characterization of cell lines.*

II. MATERIALS AND METHODS

A. CELL LINES

Four fibroblast cell lines designated as being derived from cat kidney (CK), human synovial tissue (MC for McCoy), hamster kidney (HK), and mouse (L) were used in these experiments. These cells have been maintained in serial transfers for several years in Medical Bacteriology Division. Cultures were propagated for a number of passages in either a chemically defined medium or in a lactalbumin hydrolyzate - 10% calf serum medium before their use as inocula in the respective growth medium.

B. MEDIA AND CULTURE METHODS

The compositions of the serum-free chemically defined medium and the lactalbumin hydrolyzate - 10% calf serum medium are presented in Tables 1 and 2, respectively. These are modifications of media described previously.** Media were prepared by standard procedures. Concentrated stock solutions of vitamins, antibiotics, carbohydrate, amino acids, and salts were combined and sterilized by filtration through membrane*** filters, 0.22 μ pore size.

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* Galacci, R.R.; and Galacci, R.R.; Higuchi, K. March, June, Sept. 1965. MB Division Quarterly Technical Status Reports, Nutrition Branch, Tissue Culture Section.

** Higuchi, K. December 1958. MB Division Quarterly Technical Report, Nutrition Branch.
Higuchi, K. March 1964. MB Division Triannual Report, Nutrition Branch, Tissue Culture Section.

*** Millipore Filter Corp., Bedford, Mass.

TABLE 1. SERUM-FREE CHEMICALLY DEFINED MEDIUM FOR GROWTH OF FIBROBLAST CELL LINES IN MONOLAYER CULTURE

Component	Concentration, mg/liter	Component	Concentration, mg/liter
L-Amino Acids		Salts	
Arginine·HCl	105	NaCl	7400
Asparagine	193	KCl	400
Cysteine·HCl	78	$NaH_2PO_4 \cdot H_2O$	100
Glutamine	438	$NaHCO_3$	1140[b]
Histidine·HCl	58	$CaCl_2 \cdot 2H_2O$	265
Isoleucine	131	$MgCl_2 \cdot 6H_2O$	275
Leucine	262	$Fe(NH_4)(SO_4)_2 \cdot 12H_2O$	0.97
Lysine·HCl	274		
Methionine	60	Carbon sources	
Phenylalanine	116		
Proline	200	Glucose	1800
Serine	158	Sodium pyruvate	110
Threonine	119	Gluconolactone	178
Tryptophane	61		
Tyrosine	118	Vitamins	
Valine	147		
		D-Biotin	2.5
Antibiotics and Misc.		Choline Cl	2.5
		Folic acid	2.5
Streptomycin	100	Niacinamide	2.5
Penicillin	63	Thiamine·HCl	2.5
Kanamycin	100	Ca-pantothenate	5.0
Phenol red	10	i-Inositol	2.5
Methocel, 15 cps	500	Pyridoxal·HCl	2.5
Hemin	0.30	B_{12}	0.005
N.P.H. Insulin	(5 U/l)[a]	Riboflavin	0.25

a. Units per liter.
b. Of this amount, 840 mg were added aseptically after sterilization (by filtration) and dilution of medium.

TABLE 2. LACTALBUMIN HYDROLYZATE - 10% CALF SERUM MEDIUM FOR GROWTH OF FIBROBLAST CELLS IN MONOLAYER TISSUE CULTURE

Component[a]	Concentration, mg/liter	Component	Concentration, mg/liter
Carbon and nitrogen sources		Salts	
		NaCl	7400
Lactalbumin hydrolyzate	2500	KCl	400
Glucose	2000	$NaH_2PO_4 \cdot H_2O$	100
Sodium pyruvate	110	$NaHCO_3$	972[b]
L-Glutamine	300	$CaCl_2 \cdot 2H_2O$	265
		$MgCl_2 \cdot 6H_2O$	275
Antibiotics and Misc.		Vitamins	
Streptomycin	100		
Penicillin	63	D-Biotin	2.5
Kanamycin	100	Choline Cl	2.5
Phenol red	10	Folic acid	2.5
		Niacinamide	2.5
		Thiamine·HCl	2.5
		Ca-pantothenate	5.0
		i-Inositol	2.5
		Pyridoxal·HCl	2.5
		B_{12}	0.005
		Riboflavin	0.25

a. The medium was fortified with 10% calf serum.
b. Of this amount, 672 mg were added aseptically after sterilization (by filtration) and dilution of medium.

The cells were inoculated into replicate 25 cm^2 Falcon tissue culture flasks, each containing 5 ml of medium, and incubated under static conditions at 36 C. Fresh medium was introduced daily. Cells were harvested at the intervals indicated by dispersion of monolayers with a combination of trypsin (0.01%) and EDTA (0.01 M). Duplicate 2-ml aliquots of cell suspension from each flask were centrifuged at 3,000 rpm for one minute. One sample of cells was resuspended in 2 ml of fresh growth medium to obtain the cell count in a hemocytometer. The cells in the duplicate centrifuge tube were washed and resuspended in 2 ml of the alkaline copper reagent of Oyama and Eagle[1] and stored at 5 C until protein determinations were made. Zero time protein assays of the inoculum were also made. The protein value per cell for each culture was calculated by dividing the cell protein value for the culture in µg/ml by the cell count/ml. The protein values and cell counts were then plotted and compared.

III. RESULTS

A. CHANGES IN PROTEIN CONTENT DURING THE GROWTH CYCLE

At inoculation, each flask for the experiment contained 0.30×10^5 MC cells per square centimeter of growth surface (Fig. 1). The cell protein content at zero time was 332×10^{-6} µg/cell. After one day, while in the lag phase, a maximum value of 574×10^{-6} µg/cell was obtained. The protein values then decreased to a constant minimum value by the fifth day.

A similar fluctuation in the protein contents of the CK fibroblast cell line is illustrated in Figure 2. At inoculation each flask contained 0.35×10^5 CK cells/cm^2. The protein content per cell at zero time was 179×10^{-6} µg/cell. After 2 days a maximum value of 367×10^{-6} µg/cell was obtained; then the protein value gradually decreased to a constant value by the seventh day. Also demonstrated in Figure 2 is the difference between protein values obtained by our technique and the technique of Oyama and Eagle.[1] By the latter technique in which the cells are not trypsinized, higher protein values were consistently obtained.

B. DIFFERENCES IN PROTEIN VALUES AMONG CELL LINES

The protein contents of four cell lines obtained during the latter part of the logarithmic growth in the chemically defined medium are presented in Table 3. The protein values averaged 281 (±28) $\times 10^{-6}$ µg/cell for MC cells, 219 (±17) $\times 10^{-6}$ µg/cell for HK cells, 171 (±18) $\times 10^{-6}$ µg/cell for L cells, and 134 (±18) $\times 10^{-6}$ µg/cell for CK cells. Additional data presented in Table 4 show cell line differences in protein values resulting when cells were grown in the lactalbumin hydrolyzate - 10% calf serum medium.

C. EFFECT OF GROWTH MEDIUM

A comparison of data presented in Tables 3 and 4 revealed the effects of growth medium on the protein contents of L cells, CK cells, and MC cells. A significant difference in cell protein value was noted between the two media in the case of the L cells. Protein values for L cells averaged 278 (±27) $\times 10^{-6}$ µg/cell when grown in the lactalbumin hydrolyzate - 10% calf serum medium, and 171 (±18) $\times 10^{-6}$ µg/cell when grown in the chemically defined medium. No appreciable difference in cell protein occurred in CK cells grown in the two media. The CK cell values averaged 139 (±30) $\times 10^{-6}$ µg/cell for cells grown in the serum medium and 134 (±18) $\times 10^{-6}$ µg/cell for cells grown in the defined medium. MC cells, in contrast to L cells, yielded higher protein values in the defined medium. The MC values averaged 249 (±20) $\times 10^{-6}$ µg/cell for cells grown in serum medium, and 281 (±28) $\times 10^{-6}$ µg/cell for cells in the defined medium.

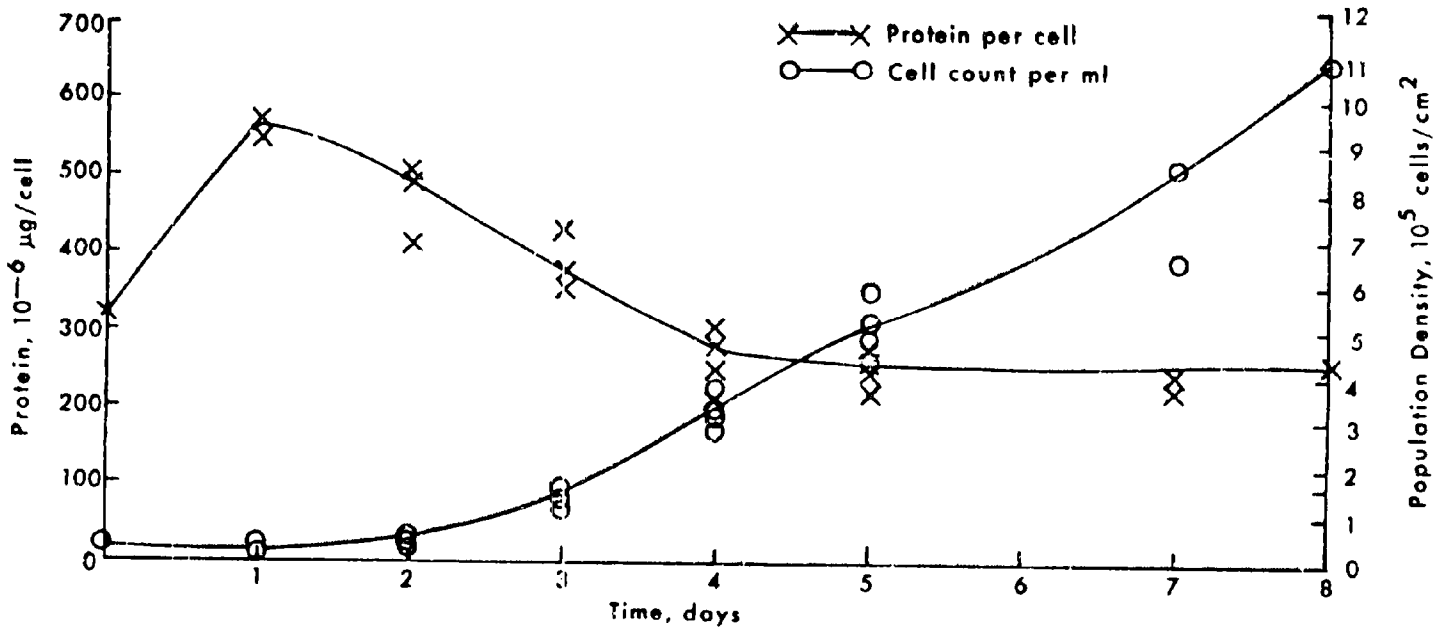

Figure 1. Changes in Protein Content During the Growth Phases of McCoy's Human Synovial Cells in a Lactalbumin Calf Serum Medium.

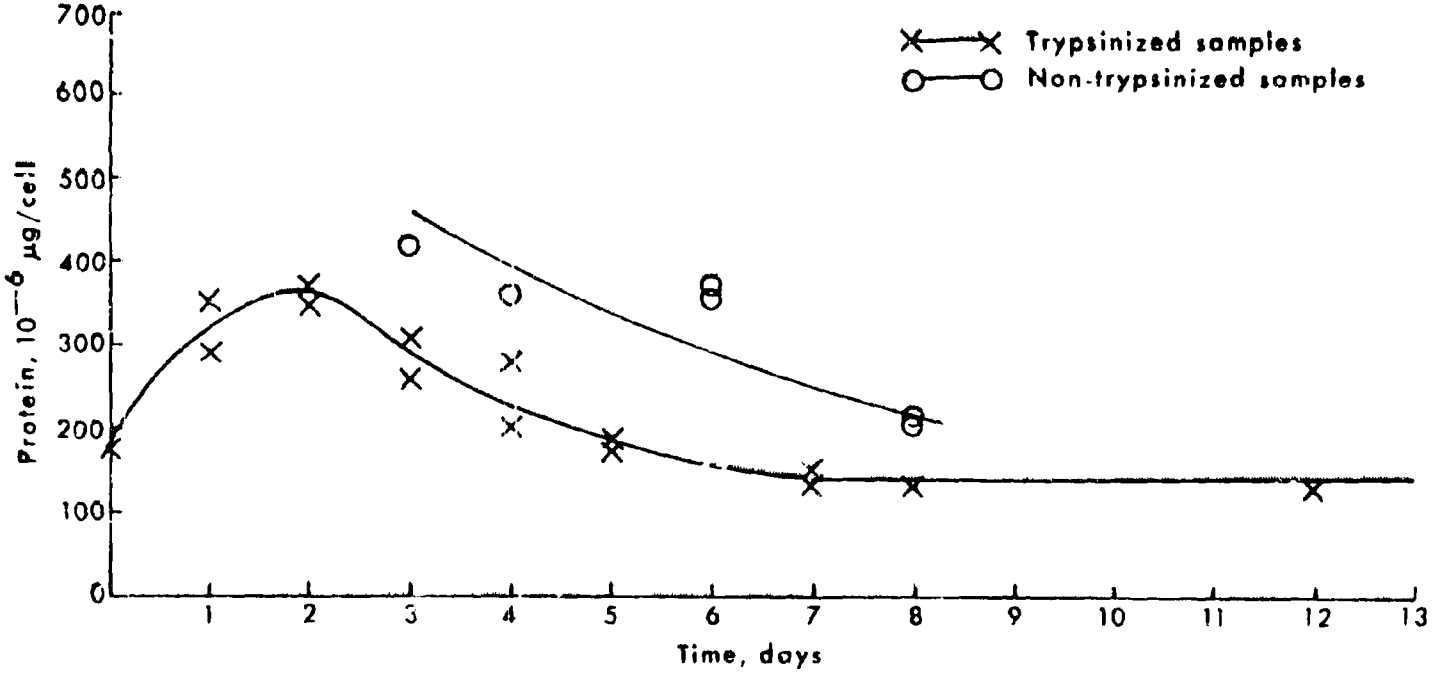

Figure 2. Changes in Protein Content During the Growth Phases of Cat Kidney Cells in a Lactalbumin Calf Serum Medium.

TABLE 3. CELL PROTEIN CONTENTS OF FIBROBLAST CELL LINES GROWN IN THE CHEMICALLY DEFINED MEDIUM

Cell Line[a]	Maximum Population,[b] 10^5 cells/cm^2	Protein,[c] 10^{-6} µg/cell	Average Protein, 10^{-6} µg/cell
Human Synovial (MC)	3	309, 273, 259, 288, 253, 303	281 (±28)
Hamster Kidney (HK)	2.2	202, 215, 231, 228	219 (±17)
Mouse (L)	15.4	167, 153, 162, 176, 171, 181, 154, 187, 188	171 (±18)
Cat Kidney (CK)	13.8	125, 138, 152, 134, 131, 139, 122	134 (±18)

a. Data from three separate experiments are presented for each cell line except HK. Only one experiment was run for HK.
b. The highest population densities reached during the determination of cell protein values.
c. The protein values were determined during the latter part of the logarithmic growth phase of the cultures.

TABLE 4. CELL PROTEIN CONTENTS OF FIBROBLAST CELL LINES GROWN IN THE LACTALBUMIN HYDROLYZATE - 10% CALF SERUM MEDIUM

Cell Line[a]	Maximum Population,[b] 10^5 cells/cm^2	Protein,[c] 10^{-6} µg/cell	Average Protein, 10^{-6} µg/cell
Mouse (L)	7.7	250, 251, 288, 297, 303, 276	278 (±27)
Human Synovial (MC)	8.1	256, 245, 249, 230, 269	249 (±20)
Cat Kidney (CK)	14.0	128, 169, 135, 131, 133	139 (±30)

a. Data from two separate experiments are presented for each cell line.
b. The highest population densities reached during the determinations of cell protein contents.
c. The protein values were determined during the latter part of the logarithmic growth phase of the cultures.

D. MAXIMUM POPULATION DENSITIES OF CELL CULTURES

The protein values reported in Tables 3 and 4 were determined when the cultures were in a slowly proliferating, densely populated, post-confluent state. The maximum population densities reached by the cells grown in the chemically defined medium were 3×10^5 cells/cm^2 for MC cells, 2.2×10^5 cells/cm^2 for HK cells, 15.4×10^5 cells/cm^2 for L cells, and 13.8×10^5 cells/cm^2 for CK cells (Table 3). The maximum population densities reached by cells grown in the lactalbumin hydrolyzate - 10% calf serum medium were 7.7×10^5 cells/cm^2 for L cells, 8.1×10^5 cells/cm^2 for MC cells, and 14×10^5 cells/cm^2 for CK cells (Table 4).

IV. DISCUSSION

We employed a modification of the method of Oyama and Eagle[1] for the determination of the protein contents of mammalian cells cultured in vitro. Swaffield and Foley[3] using a different modification of the procedures of Oyama and Eagle, and Salzman[2] employing a UV spectrophotometric method also described systematic fluctuations in protein contents of mammalian cell cultures during growth. More recently Miedema and Kruse[4] using the method described by Oyama and Eagle[1] have reported similar changes in protein values obtained with fibroblast cell lines. The protein content of cultured cells reported by all of these authors are higher than those obtained in the present work. However, it must be pointed out that in their work, different cell lines were employed; and moreover, our analytical techniques were also different. In our procedure the cells were trypsinized prior to assay for protein.

Because of the possibility that the variances described above might be attributed to a discrepancy in the protein standard used by us, comparisons were made of our protein standard (crystalline bovine serum albumin) with a commercially available protein standard (Pro-Sol),* and also with crystalline egg albumin, but no significant differences were observed. These comparisons provided assurance that our analytical standards were reliable.

The data reported in Table 3 and Table 4 show the importance of using a standard medium for the determinations of protein content per cell. The results generally varied greatly with the culture medium employed; therefore, it is recommended that a chemically defined medium be used whenever feasible in cell protein studies.

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* Standard Scientific Supply Corporation, New York, N.Y.

Because of the limited number of cell lines examined, and the clear differences in protein values among them, it was possible to identify each of the four cell lines from each other on the basis of their cell protein values. As more cell lines are examined, however, there will be overlapping of values. Nevertheless it is believed that this method can be of considerable value in many laboratories that require a method for identifying one cell type among several others of similar morphological appearance.

LITERATURE CITED

1. Oyama, V.I.; Eagle, H. 1956. Measurement of cell growth in tissue culture with a phenol reagent (Folin-Ciocalteau). Proc. Soc. Exp. Biol. Med. 91:305-307.

2. Salzman, N.P. 1958. Systematic fluctuations in the cellular protein, RNA and DNA during growth of mammalian cell cultures. Biochim. Biophys. Acta 31:158-163.

3. Swaffield, M.N.; Foley, G.E. 1959. Changes in the cellular content of ribonucleic acid, deoxyribonucleic acid and protein in cultured cells during logarithmic growth. Arch. Biochem. Biophys. 86:219-224.

4. Miedema, E.; Kruse, P.F., Jr. 1965. Enzyme activities and protein contents of animal cells cultured under perfusion conditions. Biochem. Biophys. Res. Commun. 20:528-534.